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JC14 Rec'd PCT/PTO 02 JAN 2002

<table>
<tr><td colspan="2">FORM PTO-1390 DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 1-98)
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371</td><td>ATTORNEY'S DOCKET NO.
DEBE:005US
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
10/030386</td></tr>
<tr><td>INTERNATIONAL APPLICATION NO.
PCT/DE 00/02233</td><td>INTERNATIONAL FILING DATE
July 3, 2000</td><td>PRIORITY DATE CLAIMED
July 2, 1999</td></tr>
<tr><td colspan="3">TITLE OF INVENTION
PLANTS WITH MODIFIED GENE EXPRESSION</td></tr>
<tr><td colspan="3">APPLICANT(S) FOR DO/EO/US
Koen Dekker; Martin Sagasser and Bernd Weisshaar</td></tr>
</table>

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S C. 371(c)(2)).
   a. ☒ is transmitted herewith (required only if not transmitted by the International bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: PCT Publication A2 OF WO 01/02590 comprising the application papers as filed; PCT request of July 3, 2000; PCT Publication A3 of WO 01/02590 comprising the international search report (ISR); PCT Chapter II Demand of February 2, 2001; IPER of November 15, 2001; Statement as Required Under 37 C.F.R. § 1.821(f); Diskette and Postcard.

EXPRESS MAIL MAILING LABEL
EXPRESS MAIL NO.: EL564339988US
DATE OF DEPOSIT: JANUARY 2, 2002

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/030386	INTERNATIONAL APPLICATION NO. Pct/de 00/02233	ATTORNEY'S DOCKET NUMBER DEBE 005US

17. ☒ The following fees are submitted:

CALCULATIONS PTO USE ONLY

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....$ 860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO .....$ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4).....$ 690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00

ENTER APPROPRIATE BASIC FEE AMOUNT			=	$1,000.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e))				$ .00	
Claims	Number Filed	Number Extra	Rate		
Total Claims	57 - 20 =	37	x $ 18.00	$666.00	
Independent Claims	12 - 2 =	8	x $ 80.00	$640.00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$270.00	
TOTAL OF ABOVE CALCULATIONS			=	$2,576.00	
Reduction by ½ for filing by small entity, if applicable. Applicant is entitled to small entity status pursuant to 37 CFR 1.27.				$-0-.00	
SUBTOTAL			=	$2,576.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$-0-.00	
TOTAL NATIONAL FEE			=	$-0-.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property)			+	$-0-.00	
TOTAL FEES ENCLOSED			=	$2,576.00	
				Amount to be refunded:	$ .00
				charged:	$ .00

a. ☐ A check in the amount of $_____ cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 50-1212/10112605/SLH in the amount of $ 2,576.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1212/10112605/SLH. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

STEVEN L. HIGHLANDER, ESQ.
FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
512.474.5201

SIGNATURE

Steven L. Highlander
NAME

37,642
REGISTRATION NUMBER

EXPRESS MAIL MAILING LABEL
EXPRESS MAILING NO.: EL564339998US
DATE OF DEPOSIT: JANUARY 2, 2002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Keon Dekker; Martin Sagasser and Bernd Weisshaar

Serial No.: Unknown

Filed: January 2, 2002

For: PLANTS WITH MODIFIED GENE EXPRESSION

Group Art Unit: Unknown

Examiner: Unknown

Atty. Dkt. No.: DEBE:005US/SLH

PRELIMINARY AMENDMENT

Hon. Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please consider the following amendments prior to examination of the above-captioned application. It is believed that no fees are occasioned by this filing; however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Commissioner is authorized to deduct said fees from Fulbright & Jaworski L.L.P. Account No.: 50-1212/10112605/DEBE:005US. Please date stamp and return the enclosed postcard as evidence of receipt.

AMENDMENT

In the Specification

Please insert the following paragraph after line 1 of page 1:

This application is a national stage filing under 35 U.S.C. §371 of PCT/de 00/02233, filed on July 3, 2000, which claims priority to German DE 199 30 570.6, filed July 2, 1999. The entire content of both these applications are incorporated by reference.

In the Claims

Please amend claims 1-30 as follows:

1. (Amended) A method for producing a plant with modified gene expression comprising (i) stable integration into the genome of plant cells or plant tissues of (a) a seed specific regulatory sequence or a fragment or derivative thereof, provided said fragment or derivative controls specifically the expression of genes in the seed, and (b) a nucleic acid sequence encoding a gene product, wherein the nucleic acid sequence is functionally linked to said seed specific regulatory sequence or the fragment or derivative thereof, and (ii) regeneration of the obtained plant cells or plant tissues to plants.

2. (Amended) The method according to claim 1, wherein said gene expression is enhanced or reduced.

3. (Amended) The method according to claim 1, wherein said nucleic acid sequence encoding a gene product is an endogeneous or exogeneous nucleic acid sequence.

4. (Amended) The method according to claim 1, wherein said nucleic acid sequence encoding a gene product a nucleic acid sequence is selected from the group consisting of genes of the phenyl propanoid metabolism, seed specific genes, seed coat-specific genes and genes of the general metabolism.

5. (Amended) The method according to claim 4, wherein said genes of the phenylpropanoid metabolism are selected from the group consisting of genes for phenylalanine ammonia-lyase, cinnamate 4-hydroxylase, 4-coumarate-coA ligase, chalcone synthase, chalcone isomerase, chalcone reductase, flavanone 3-hydroxylase, flavonoid-3'-hydroxylase, flavonoid-3'5'-

hydroxylase, dihydroflavono-4-reductase, leucoanthocyanidin reductase, leucoanthocyanidin dioxygenase, 3'-glucoysltransferase, 5'-glujcosyltransferase and O-methyl transferase.

6. (Amended) The method according to claim 4, wherein said seed-specific genes are selected from the group consisting of genes influencing germ tendency or dormancy, pathogen resistance, or the TT1 gene according to SEQ ID NO:2 and SEQ ID NO:4.

7. (Amended) The method according to claim 4, wherein said genes of the general metabolism are selected from the group consisting of genes for ADP glucose synthethase, starch synthase, ADP glucose pyrophosphorylase and yeast invertase.

8. (Amended) The method according to claim 1, wherein said seed-specific regulatory sequence comprises SEQ ID NO:1 or a fragment or derivative thereof.

9. (Amended) A transformed plant cell or transformed plant tissue, characterized in that (i) a seed specific regulatory sequence, or a fragment or derivative thereof, and (ii) a nucleic acid sequence encoding a gene product, wherein the nucleic acid sequence is functionally linked to said seed specific regulatory sequence or a fragment or derivative thereof, are stably incorporated into the genome of said plant cell or said plant tissue.

10. (Amended) A nucleic acid segment comprising the sequence according to SEQ ID NO:1.

11. (Amended) A fragment or derivative of the nucleic acid sequence according to SEQ ID NO:1, or a nucleic acid sequence which hybridizes with the nucleic acid sequence according SEQ ID NO:1, said fragment, derivative or hybridizing nucleic acid sequence being responsible for the seed specific expression.

12. (Amended) The hybridizing nucleic acid sequence according to claim 11, wherein the hybridizing nucleic acid sequence hybridizes with the nucleic acid sequence according SEQ ID NO:1 under stringent conditions.

13. (Amended) A method for producing plants with modified flavonoid content, comprising (i) stable integration of a nucleic acid segment comprising the nucleic acid sequence according to SEQ ID NO:2 or 4 or a homologous nucleic acid sequence thereto, or of a fragment or derivative thereof with the biological activity of a polypeptide encoded by the nucleic acid sequence according to SEQ ID NO:2 or 4, into the genome of plant cells or plant tissues, and (ii) regeneration of the obtained plant cells or plant tissues to produce plants.

14. (Amended) The method according to claim 13, whereby the integrated nucleic acid sequence or a fragment or derivative thereof is transcribed in sense or antisense orientation compared to the endogenous nucleic acid sequence.

15. (Amended) The method according to claim 13, wherein the formation of flavonoids is inhibited by a ribozyme, comprising the integrated nucleic acid sequence or a fragment or derivative thereof.

16. (Amended) The method according to claim 13, wherein the nucleic acid sequence or a fragment of derivative thereof is integrated into the genomic region of the homologous endogenous gene by homologous recombination.

17. (Amended) The method according to claim 13, wherein the nucleic acid sequence or a fragment or derivative thereof is functionally linked to a regulatory DNA sequence, which controls the expression of the integrated nucleic acid sequence or a fragment or derivative thereof.

18. (Amended) The method according to claim 17, wherein the regulatory DNA sequence is selected from the group of promoters CaMV 35S Promoter, PRPI promoter, phaseolin promoter, isoflavon reduktase promoter, ST-LSI promoter, salicylic acid-inducible promoter, benzenesulfonamide-inducible promoter, tetracycline-inducible promoter, abscisic acid-inducible promoter, ethanol- or cyclohexanon-inducible promoter, promoter according to SEQ ID NO:1 or a seed specific promoter from tobacco.

19. (Amended) A nucleic acid segment comprising the sequence according to SEQ ID NO:2 or 4.

20. (Amended) A fragment or derivative of the nucleic acid sequence according to SEQ ID NO:2 or 4, or a nucleic acid sequence which hybridizes to the nucleic acid sequence according to SEQ ID NO:2 or 4 and is responsible for the formation of flavonoids.

21. (Amended) The hybridizing nucleic acid sequence according to claim 20, wherein the hybridizing nucleic acid sequence hybridizes under stringent conditions with the nucleic acid sequence according to SEQ ID NO:2 or 4.

22. (Amended) A transformed plant cell or transformed plant tissue, characterized in that the nucleic acid sequence selected from SEQ ID NO:2, SEQ ID NO:4, or a fragment, derivative or homolog thereof, or a nucleic acid that hybridizes to SEQ ID NO:2 or SEQ ID NO:4 is stably integrated into the genome of the plant cell or plant tissue.

23. (Amended) A polypeptide comprising the amino acid sequence of SEQ ID NO:3.

24. (Amended) A plant cell or plant tissue according to claim 9 or claim 22, regenerable to a seed producing plant.

25. (Amended) A plant obtainable according to any one of claims 1 to 8 or 13 to 18.

26. (Amended) A seed obtained by plants according to claim 25.

27. (Amended) A vector comprising a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, or a fragment, derivative or homolog thereof, or a nucleic acid that hybridizes to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4.

28. (Amended) A transgenic plant, cells of which comprise (i) a stably integrated seed specific regulatory nucleic acid sequence according to SEQ ID NO:1, or a fragment or derivative

or homolog thereof with the biological function of a seed specific promoter, and (ii) a nucleic acid sequence encoding a gene product functionally linked to said seed specific regulatory nucleic acid sequence.

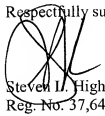
29. (Amended) A transgenic plant, cells of which comprise a stably integrated nucleic acid sequence according to SEQ ID NO:2 or 4, or a homologous nucleic acid sequence thereto, or a fragment or derivative thereof with the biological activity of a polypeptide encoded by the nucleic acid sequence according to SEQ ID NO:2 or 4.

30. (Amended) A transgenic plant according to claim 29, wherein the nucleic acid sequence or a fragment or derivative thereof is functionally linked to a regulatory DNA sequence which controls the expression of the integrated nucleic acid sequence or a fragment or derivative thereof.

REMARKS

Should the examiner have any questions regarding the content of this preliminary amendment, a telephone call to the undersigned is invited.

Respectfully submitted,



Steven L. Highlander
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Attorney for Steven Donovan

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600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3184

Date: January 2, 2002

APPENDIX A: MARKED UP COPY OF CLAIMS

1. (Amended) A method for producing a plant with modified gene expression comprising (i) stable integration into the genome of plant cells or plant tissues of (a) a seed specific regulatory sequence or a fragment or derivative thereof, provided said fragment or derivative controls specifically the expression of genes in the seed, and (b) a nucleic acid sequence encoding a gene product, wherein the nucleic acid sequence is functionally linked to said seed specific regulatory sequence or the fragment or derivative thereof, [into the genome of plant cells or plant tissues] and (ii) regeneration of the obtained plant cells or plant tissues to plants.

2. (Amended) [Method] The method according to claim 1, wherein said gene expression is enhanced or reduced.

3. (Amended) [Method] The method according to claim 1 [or 2], wherein [for] said nucleic acid sequence encoding a gene product is an endogeneous or exogeneous nucleic acid sequence [is used].

4. (Amended) [Method] The method according to [anyone claims 1-3] claim 1, wherein [for] said nucleic acid sequence encoding a gene product a nucleic acid sequence is selected from the group consisting of genes of the phenyl propanoid metabolism, seed specific genes, seed coat-specific genes [or] and genes of the general metabolism [is used].

5. (Amended) [Method] The method according to claim 4, wherein [for] said genes of the phenylpropanoid metabolism [a nucleic acid sequence] are selected from the group consisting of genes for phenylalanine ammonia-lyase, cinnamate 4-hydroxylase, 4-coumarate-coA ligase, chalcone synthase, chalcone isomerase, chalcone reductase, flavanone 3-hydroxylase, flavonoid-3'-hydroxylase, flavonoid-3'5'-hydroxylase, dihydroflavono-4-reductase, leucoanthocyanidin reductase, leucoanthocyanidin dioxygenase, 3'-glucoysltransferase, 5'-glujcosyltransferase and O-methyl transferase.

6. (Amended) [Method] The method according to claim 4, wherein [for] said seed-specific genes [a nucleic acid sequence is used] are selected from the group consisting of genes influencing germ tendency or dormancy, [or] pathogen resistance, or the TT1 gene according to SEQ ID NO:2 and SEQ ID NO:4.

7. (Amended) [Method] The method according to claim 4, wherein [for] said genes of the general metabolism [a nucleic acid sequence is used] are selected from the group consisting of genes for ADP glucose synthethase, starch synthase, ADP glucose pyrophosphorylase and yeast invertase.

8. (Amended) [Method] The method according to [anyone of claims 1 to 7] claim 1, wherein [for] said seed-specific regulatory sequence [the nucleic acid sequence according to] comprises SEQ ID NO:1 or a fragment or derivative thereof [is used].

9. (Amended) [Transformed] A transformed plant cell or transformed plant tissue, characterized in that (i) a seed specific regulatory sequence, or a fragment or derivative thereof, and (ii) a nucleic acid sequence encoding a gene product, wherein the nucleic acid sequence is functionally linked to said seed specific regulatory sequence or a fragment or derivative thereof, [is stable] are stably incorporated into the genome of [the] said plant cell or [the] said plant tissue.

10. (Amended) [Nucleic] A nucleic acid segment comprising the sequence according to SEQ ID NO:1.

11. (Amended) [Fragment] A fragment or derivative of the nucleic acid sequence according to [claim 10] SEQ ID NO:1, or a nucleic acid sequence which hybridizes with the nucleic acid sequence according SEQ ID NO:1, [and is] said fragment, derivative or hybridizing nucleic acid sequence being responsible for the seed specific expression.

12. (Amended) [Nucleic] The hybridizing nucleic acid sequence according to claim 11, wherein the hybridizing nucleic acid sequence hybridizes with the nucleic acid sequence according SEQ ID NO:1 under stringent conditions.

13. (Amended) [Method] A method for producing plants with modified flavonoid content, comprising (i) stable integration of [at least of] a nucleic acid segment comprising the nucleic acid sequence according to SEQ ID NO:2 or 4 or a homologous nucleic acid sequence thereto, or of a fragment or derivative thereof with the biological activity of a polypeptide encoded by the nucleic acid sequence according to SEQ ID NO:2 or 4, into the genome of plant cells or plant tissues, and (ii) regeneration of the obtained plant cells or plant tissues to produce plants.

14. (Amended) [Method] The method according to claim 13, whereby the integrated nucleic acid sequence or a fragment or [derivate] derivative thereof is [expressed] transcribed in sense or antisense orientation compared to the endogenous nucleic acid sequence.

15. (Amended) [A] The method according to claim 13 [or 14], wherein the formation of flavonoids is inhibited by a ribozyme, comprising the integrated nucleic acid sequence or a fragment or derivative thereof.

16. (Amended) [Method] The method according to claim 13 [or 14], wherein the nucleic acid sequence or a fragment of derivative thereof is integrated into the genomic region of the homologous endogenous gene by homologous recombination.

17. (Amended) [Method] The method according to [anyone of claims] claim 13 [to 16], wherein the nucleic acid sequence or a fragment or derivative thereof is functionally linked to a regulatory DNA sequence, which controls the expression of the integrated nucleic acid sequence or a fragment or derivative thereof.

18. (Amended) [Method] The method according to claim 17, wherein the regulatory DNA sequence is selected from the group of promoters CaMV 35S Promoter, PRPI promoter, phaseolin promoter, isoflavon reduktase promoter, ST-LSI promoter, salicylic acid-inducible

promoter, benzenesulfonamide-inducible promoter, tetracycline-inducible promoter, abscisic acid-inducible promoter, ethanol- or cyclohexanon-inducible promoter, promoter according to SEQ ID NO:1 or a seed specific promoter from tobacco.

19. (Amended) [Nucleic] A nucleic acid segment comprising the sequence according to SEQ ID NO:2 or 4.

20. (Amended) [Fragment] A fragment or derivative of the nucleic acid sequence according to SEQ ID NO:2 or 4, or a nucleic acid sequence which hybridizes to the nucleic acid sequence according to SEQ ID NO:2 or 4 and is responsible for the formation of flavonoids.

21. (Amended) [Nucleic acid sequence] The fragment or derivative according to claim 20, wherein the hybridizing nucleic acid sequence hybridizes under stringent conditions with the nucleic acid sequence according to SEQ ID NO:2 or 4.

22. (Amended) [Transformed] A transformed plant cell or transformed plant tissue, characterized in that the nucleic acid sequence [according to anyone of claims 19 to 21] selected from SEQ ID NO:2, SEQ ID NO:4, or a fragment, derivative or homolog thereof, or a nucleic acid that hybridizes to SEQ ID NO:2 or SEQ ID NO:4 is [stable] stably integrated into the genome of the plant cell or plant tissue.

23. (Amended) [Amino] A polypeptide comprising the amino acid sequence [as listed in] of SEQ ID NO:3.

24. (Amended) [Plant] A plant cell or plant tissue according to claim 9 or claim 22, regenerable to a seed producing plant.

25. (Amended) [Plant] A plant obtainable according to [anyone] any one of [claim] claims 1 to 8 or 13 to 18.

26. (Amended) [Seeds] A seed obtained by plants according to claim 25.

27. (Amended) [Vector,] A vector comprising a nucleic acid sequence [according to anyone of claims 10 to 12 or 19 to 21] selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, or a fragment, derivative or homolog thereof, or a nucleic acid that hybridizes to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4.

28. (Amended) [Transgenic] A transgenic plant [with a stable into the genome], cells of which comprise (i) a stably integrated seed specific regulatory nucleic acid sequence according to SEQ ID NO:1, or a fragment or derivative or homolog thereof with the biological function of a seed specific promoter, and (ii) a nucleic acid sequence encoding a gene product functionally linked to said seed specific regulatory nucleic acid sequence.

29. (Amended) [Transgenic] A transgenic plant [with a stable into the genome], cells of which comprise a stably integrated nucleic acid sequence according to SEQ ID NO:2 or 4, or a homologous nucleic acid sequence thereto, or a fragment or derivative thereof with the biological activity of a polypeptide encoded by the nucleic acid sequence according to SEQ ID NO:2 or 4.

30. (Amended) [Transgenic] A transgenic plant according to claim 29, wherein the nucleic acid sequence or a fragment or derivative thereof is functionally linked to a regulatory DNA sequence which controls the expression of the integrated nucleic acid sequence or a fragment or derivative thereof.

3/pats

PCT/DE 00/02233

Plants with modified gene expression

The present invention relates to a method for producing a plant with modified gene expression, comprising the stable integration of a seed-specific regulatory sequence or fragment or derivative thereof and a nucleic acid sequence that is functionally linked to said seed-specific regulatory sequence or fragment or derivative thereof, encoding a gene product, in the genome of plant cells or plant tissues, and the regeneration of the resulting plant cells or plant tissues to plants. The present invention also relates to a method for producing plants with a modified flavonoid content, comprising the stable integration of at least the nucleic acid sequence according to SEQ ID NO:2 or 4 or a nucleic acid sequence that is homologous with this, or fragment or derivative thereof in the genome of plant cells or plant tissues, and the regeneration of the resulting plant cells or plant tissues to plants.

The phenylpropanoid pathway of the plant, reviewed e.g. by Weisshaar & Jenkins, Current Opinion in Plant Biology 1, 251-257 (1998) or by Shirley, Trends in Plant Sciences 1, 377-382 (1996), consists of a complex series of branching biochemical reactions which provide the plant with varied phenolic compounds. The identical start of all following synthesis leads from phenylalanine via cinnamate and 4-coumarate to coumaroyl-CoA. The enzymes phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H) and 4-coumarate CoA-ligase (4CL) are involved. In the further course the synthesis branches. An important branch leads to the biosynthesis of flavonoids. Flavonoids are flavan derivatives, i.e. secondary metabolites that are unique to plants with a flavan skeleton comprising two aromatic rings. To the many flavonoids, mostly specific for a special plant, belong amongst others the UV-absorbing flavonols, to the tanning-agents belonging tannins and anthocyanins formed e.g. as blue or red flower pigments.

In *Arabidopsis thaliana* several different chromosomal gene loci are known, which play a role in the flavonoid biosynthesis. Mutations in a number of these loci prevent the accumulation of brown pigments in the seed coat (testa) and are named transparent testa (tt in mutated form, TT as wild-type). Because of the cotelydons under the seed coat the seed looks yellow to pale-brown. Wild-type seeds in contrast are dark brown. Some of these loci (tt3, tt4, tt5, ttg) in addition take part in the production of anthocyanins in leaves and stems; one locus (ttg) plays additional roles in trichome and root hair development. All so far occuring tt-mutants were

shown to be recessive. A recapitulating description is found in Shirley et al., The Plant Journal 8, 659-671 (1995).

The first Arabidopsis mutants with a defect in flavonoid biosynthesis were identified 1971 by Bürger (Bürger, Arabidopsis Information Service 8, 36-42 (1971)). In this publication the phentoype of tt1, which shows a different seed colour, was mentioned the first time. Because of genetic and morphological studies by Koornneef (Koornneef, Arabidopsis Information Service 18, 45-51 (1981), Koornneef, Arabidopsis Information Service 27, 1-4 (1990) as well as by Shirley (Shirley et al., The Plant Journal 8, 659-671 (1995) the gene locus of tt1 could be located on chromosome 1 –54,9, and the phenotype could be restricted to the seeds.

A change in seed composition and of the flavonoid biosynthesis is desired in many useful plants and ornamental plants for a long time in point of view of agriculture and production. A limited influence on components of the flavonoid metabolism was only possible by classical breeding methods, as long as the gene structure of the involved enzymes was not known. This conventional method, based on the random blending of the maternal and paternal heritable information, is relatively time- and cost-consuming. The desired result is to be expected only after 10 to 15 years. The targeted manipulation of individual components of the flavonoid biosynthesis is hardly achieved by classical breeding without genetic analyses. It is therefore an object to provide plants with a change in seed composition and an improvement in seed quality of plants, e.g. useful plants and ornamental plants.

The objective of the invention is achieved by the matter of the claims.
The invention is further described by reference to the following non-limiting figures.

Figure 1 shows a schematic representation of the analysis of the nucleic acid sequence of the TT1-promoter in comparison with TRANSFAC MATRIX TABLE, Rel.3.3 (E. Wingender et al., 1998). Binding sites for transcription factors are indicated by capital letters (SBF-1 like sites: see Lawton et al, Silencer region of a chalcone synthase promoter contains multiple binding sites for a factor, SBF-1, closely related to GT-1, Plant Molecular Biology 16, 235-249 (1991)), in italics (AGAMOUS like sites: Huang et al., isolation and characterization of the binding sequences for the product of the Arabidopsis floral homeotic gene AGAMOUS, Nucleic Acids Research 21, 4769-4776 (1993)), underlined letters (P like sites: Grotewold et al., The myb-homologous P

gene controls phlobabene pigmentation in maize floral organs by directly activating a flavonoid biosynthetic gene subset, Cell 76, 543-553 (1994)), capital and italic letters (MYB Ph3 like sites: Solano et al., Dual DNA binding specifity of a petal epidermis-specific MYB transcription factor (MYB.PH3) from Petunia hybrida, EMBO Journal 14, 1773-1784 (1995)) as well as capital and underlined letters (Athab-1 und 2 like sites: Sessa et al., The athb-1 and -2 HD-Zip domains homodimerize forming complexes of different DNA binding specificities, EMBO Journal 12, 3507-3517 (1993)). The start-ATG is indicated by bold and underlined letters. Numbering starts with the 5' SpeI restriction site in the used plasmid vector pSK-TT1.

Figure 2 shows the nucleic acid sequence of the genomic DNA sequence of TT1, commencing with the start-ATG. Capitals represent exons, introns are in italics. Numbering resumes the one of figure 1.

Figure 3 shows the cDNA sequence, encoding the TT1 gene and the predicted amino acid sequence of TT1.

Figure 4 shows schematically an alignment of the amino acid sequence of TT1 with sequences of the NCBI GenBank. acc. No AL049660, AB025629 and AC006085.9 are hypothetical amino acid sequences derived from nucleic acid sequences of *Arabidopsis thaliana* (At), AJ234704 is a hypothetical amino acid sequence derived from a nucleic acid sequence of *Hordeum vulgare* (Hv). In the consensus sequence a ! denotes amino acids of the type I or V, a $ amino acids of the type L or M, a % amino acids of the type F or Y and a # amino acids of the type N, D, Q, E, B or Z.

Figure 5 shows schematically the restriction map of pSK-TT1.

Figure 6 shows a representation of the seed colour of the mutant tt1 in comparison to the wild-type.

Figure 7 shows a representation of the nuclear localisation of TT1.

Figure 8 shows X-Gluc stained flowers and siliques of transgenic *TT1*-GUS plants in different developmental stages. A shows a flower shortly after pollination. GUS-activity is restricted to

the apical ovules. More mature flowers (B) and young siliques (F) show GUS-activity in funiculi and integuments of apical and distal ovules (C and D). With the used method, GUS-activity is detectable in ovules, which are not developing further, but not in older seed stages (E).

The term „homologous sequence" or „homologous nucleic acid sequence" or "homologue" as used herein refers to a nucleic acid or protein sequence with significant similarity to the compared sequence or parts thereof, whereby this homologous sequences show an activity or part-activity comparable to the activity of the nucleic acid sequence or protein sequences according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4. As homologous sequences are considered nucleic acid sequences, which hybridize with the sequences according to SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:4 or parts of these sequences under stringent or less stringent conditions (about stringent and less stringent conditions see Sambrook et al., Molecular Cloning, Cold Spring Harbour Laboratory (1989), ISBN 0-87969-309-6). An example for stringent hybridization condition is: hybridization in 4 x SSC at 65° C (alternatively in 50% formamide and 4 X SSC at 42° C), followed by several washing steps in 0,1 x SSC at 65°C for one hour. An example for less stringent hybridization condition is hybridization in 4 x SSC at 37° C, followed by several washing steps in 1 x SSC at room temperature. As homologous sequences are furthermore considered nucleic acid or protein sequences or parts thereof, which show a significant similarity with the nucleic acid and amino acid sequences of the present invention using the similarity algorithm BLAST (Basic Local Alignment Search Tool, Altschul et al., Journal of Molecular Biology 215, 403-410 (1990). As significant similar, as used herein, are considered sequences, which e.g. using standard parameters in Blast-Service of NCBI show a Probability of $P < 10^{-5}$, if they are compared with the sequences according to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4 or parts thereof.

The term "derivative" as used herein refers to nucleic acid sequences, which show one or several deletions, substitutions, additions, insertions and/or inversions.

The term "functionally linked" as herein used means, that a regulatory sequence as a promoter controls the expression of a gene or that a nucleic acid sequence is expressed based on the promoter.

The term "vector" as used herein refers to natural occurring or artificial created constructs for

incorporation, propagation, expression or transfer of nucleic acids, e.g. plasmids, phagemids, cosmids, artificial chromosoms, bacteriophages, viruses, retroviruses.

The term „expression system" as used herein refers to any combination of vectors, restriction enzymes, transformation methods, cell extracts, living cells e.g. procaryotic or eucaryotic cells or organisms for the purpose of endogenous or exogenous expression of genes.

The present invention relates to the regulatory seed-specific nucleic acid sequence (below also referred to as promoter), which naturally controls the expression of the TT1-gene in *Arabidopsis thaliana*. The nucleic acid sequence according to the invention is listed in SEQ ID NO:1. Furthermore the invention relates to a fragment or derivative of the nucleic acid sequence according to SEQ ID NO:1 or a nucleic acid sequence, which hybridizes with nucleic acid sequence according to SEQ ID NO:1 and is responsible for the seed-specific expression. Preferred is a nucleic acid sequence, which hybridizes under stringent conditions with the nucleic acid sequence according to SEQ ID NO:1.

The nucleic acid sequence according to the invention may be of natural origin or be artificial constructed. The nucleic acid sequence may exist in sense as well as in antisense orientation.

The nucleic acid sequence according to the invention may be used e.g. for the identification and isolation of homologous genes to the TT1-gene from other organisms or of homologous genes from *Arabidopsis thaliana* using special hybridization or screening procedures, e.g. as probe for the screening in DAN libraries with the help of hybridizing to single stranded nucleic acids of similar base sequence.

The nucleic acid sequence according to the invention may further be used for specific control of the expression of genes in organisms or cells, preferable for specific control of the expression of genes in seed, particularly in the seed coat. Preferably the present invention provides a method to produce a plant with modified gene expression, comprising the stable integration of a seed-specific regulatory sequence or a fragment or derivative thereof and a nucleic acid sequence, functionally linked to said seed-specific regulatory sequence or fragment or derivative thereof, encoding a gene product, into the genome of plant cells or plant tissues and regeneration of the resulting plant cells or plant tissues to plants. The nucleic acids functionally linked to the nucleic

acid according to the present invention may be endogenous, exogenous genomic DNA segments or cDNAs or fragments or derivatives thereof. Endogenous means, that the nucleic acid sequence is derived from the same organism in which it will be integrated by the method according to the present invention, e.g. a nucleic acid sequence of *Arabidopsis thaliana* is integrated into *Arabidopsis thaliana* with the method according to the invention. Exogenous means, that the nucleic acid sequence is derived from a different organism, e.g. a nucleic acid sequence of *Arabidopsis thaliana* is integrated in e.g. wheat by the method according to the invention. The nucleic acid sequences may show deletions, substitutions, additions, insertions and/or inversions compared to the naturally occurring nucleic acid sequences.

The nucleic acid sequence according to the invention may be used in vectors, expression systems or plants, plant tissues or plant cells or animal cells or microorganisms to modify the expression profile of various gene products. The expression of the gene products may be up-regulated as well as down-regulated compared to the natural expression.

For instance the nucleic acid sequence according to the invention may be used for the expression of the seed coat specific TT1-gene. Furthermore the nucleic acid sequence according to the invention may be suited for the regulation of the expression of other gene sequences for any use, both from *Arabidopsis thaliana* as from different organisms. Thereby the promoter may be present in combination with any genes in the vector as well as in transgenic organisms.

In particular the nucleic acid sequence according to the present invention may be utilized for the control of expression of further natural seed-specific or artificially into the seed transferred genes, e.g. for regulation of expression of further genes of the phenylpropanoid metabolism, e.g. phenylalanine ammonia-lyase, cinnamate 4-hydroxylase, 4-coumarate coenzyme A ligase, chalcone synthase, chalcone isomerase, chalcone reductase, flavanone 3-hydroxylase, flavonoid 3'-hydroxylase, flavonoid 3',5'-hydroxylase, dihydroflavonol 4-reductase, leucoanthocyanidine reductase, leucoanthocyanidine dioxygenase, 3'-glucosyltransferase, 5'-glucosyltransferase, O-methyltransferase. The control of expression of genes of the phenylpropanoid metabolism using the nucleic acid sequence according to the present invention may be useful in particular e.g. for enhancement of the UV-absorption rate, modification of colour, improvement of taste or shelf life, enhancement of protection against varmints or improvement of processing of various plant tissues.

Furthermore the nucleic acid sequence according to the present invention may be used for regulation of expression of genes, encoding further proteins of the seed coat. The proteins may improve the quality, i.e. the composition of the seeds and/or alter the quality, i.e. their physiological characteristics. Examples for preferred proteins of the seed coat are proteins, which influence germ tendency or dormancy. Dormancy is caused respectively controlled in many cases by various substances of the seed coat. A alteration of dormancy may be achieved by using the nucleic acid sequence of the present invention for regulation of genes expressed in the seed coat and influencing germ tendency or dormancy. Such genes are e.g..

a) genes, which take part in formation of waterproof layers (e.g. waxen cuticulae or suberin lamellae), e.g. GenBank acc. no. AF030260 (cytochrom P450 CYP94A1), GenBank acc. no. M80567 (lipid transfer protein LTP1),

b) genes for the synthesis of seed coat components, which oppose a mechanical resistance to the embryo such as lignins, e.g. GenBank acc. no. J02979 (lignin forming peroxidase),

c) genes for proteins, which weaken the mechanical resistance of the seed coat against the embryo, such as cell wall components digesting enzymes, e.g. GenBank acc. no. AJ242807 (cellulase), GenBank acc. no. AJ277900 (beta 1,3-glucanase),

d) genes for synthesis of growth inhibitors such as abscisic acid, e.g. GenBank acc. no. U95953 (viviparous 14), GenBank acc. no. AF190462 9-(cis-epoxycarotenoid dioxygenase),

e) genes for synthesis of seed coat components, which hold back growth inhibitors such as abscisic acid in the seed coat,

f) genes for synthesis of seed coat components, which influence the gas exchange and thereby the oxygen supply of the embryo,

g) genes for components of the secondary metabolism, which influence seed vitality.

Further examples for preferred proteins of the seed coat are proteins, which directly or indirectly mediate seed resistance against pathogen attack by insects, fungi, bacteria, viruses or nematodes. Various classes of proteins and secondary metabolites take part in corresponding defense mechanisms. In this sense an improved pathogen resistance may be achieved by utilizing the nucleic acid sequence according to this invention for the regulation of genes expressed in the seed coat and taking part in pathogen defense. Such genes are e.g.

a) genes for insecticide effective α–amylase inhibitors, proteinase inhibitors and fiber proteins, e.g. GenBank acc. no. D26109 (alpha-amylase inhibitor-2), GenBank acc. no. AF105340 (proteinase inhibitor precursor),

b) genes for synthesis of polymeric cell wall components such as callose, which serve as physical barrier against fungi and bacteria infections, e.g. GenBank acc. no. AF085717 (callose synthase catalytic subunit)

c) genes for hydrolytic enzyme such as glucanases and chitinases, which resolve the cell walls of the pathogen, e.g. GenBank acc. no. AF241267 (chitinase 2),

d) genes for synthesis of antimicrobially acting phytoalexins, e.g. GenBank acc. no. U69554 (6a-hydroxymaackiain methyltransferase)

e) genes from the group of plant R-genes (resistance genes), whose gene products interact directly or indirectly via further proteins with gene products of avr-genes (avirulence genes) of the pathogen and lead to programmed cell death of infected plant cells at the infection place, (hypersensitive response e.g.). GenBank acc. no. BE039015 (downy mildew resistance protein genes rpp5), GenBank acc. no. AF122994 (RPM1 variant genes), AF098962, GenBank acc. no. AF122994 (RPM1 variant genes), GenBank acc. no. AF098962 (disease resistance protein RPP1-WsA genes)

f) genes encoding proteins with DNA-binding WRKY domains, which through binding to so called W boxes activate pathogen defence genes, e.g. GenBank acc. no. AF193770 (WRKY 3), GenBank acc. no. AF193771 (WRKY 4),

g) genes for saponines, which can destroy the fungi membrane because of binding to sterols.

In addition, the nucleic acid sequence according to the invention may be useful for the improvement of the nutritive value and the digestibility of the seed. A corresponding modification may be achieved e.g. by a decrease of the raw fiber content, a reduction in antinutritive substances or a modification in the content of proteins and storage lipids, by using the nucleic acid sequence of the invention for regulation of genes participating in these processes.

Furthermore, the nucleic acid sequence according to the present invention is suited for the regulation of storage compounds in the seed, e.g. of starch. Possibilities of influencing storage compounds exist e.g. in expressing sense or antisense transcripts of the carbohydrate metabolism

of the plant such as ADP glucose synthetase, starch synthase, ADP glucose pyrophosphorylase or expression of genes of different organisms such as yeast invertase for the mobilisation of starch.

The nucleic acid sequence of the present invention may further be useful for the relocation of metabolism products of the seed into the seed coat, e.g. glucosinolates for improvement of resistance against nematodes. Furthermore, the promoter may be used for prevention or delay of ripening of the seed coat through controlled expression of ribonuclease genes. A delay in ripening of the seed coat may lead to formation of bigger seeds. In addition the removal of the seed coat may be facilitated by a delayed ripening.

Suitable vectors for integration and transfer of the nucleic acid sequence of the present invention and of a functionally linked nucleic acid sequence encoding a gene product may ensure the propagation and/or expression of the integrated nucleic acids in procaryotes such as *Escherichia coli* or *Agrobacterium tumefaciens* or in plant cells, plant tissues or plants or animal cells or animals. Corresponding vectors may occur naturally or be produced artificially. The vectors may comprise selection markers, terminator sequences, polylinker, promoter elements, enhancer, polyadenylation sites and other genetic elements. Suitable vectors for cloning are e.g. pBluescript, plasmids of the pUC-series, plasmids of the pGem-series or on bacteriophage λ based vectors. A plasmid vector for use in Agrobacterium is e.g. pBin19 (Bevan et al., Nucleic Acids Research 12, 8711-8721. (1984)). For the transformation and expression in plants, construct based on Ti-plasmids of Agrobacterium species or plant viruses represent usable vectors and are known to those skilled in the art. A reviewing description of previously used vectors can be found by Guerineau and Mullineaux, Plant Transformation and Expression Vectors, in: Plant Molecular Biology Labfax, edited by Croy, Oxford, BIOS Scientific Publishers, 121-148 (1993).

Some of the in commercial transformation and expression systems used methods of transformation for the transfer of foreign genes (transformation) into the genome of plants are introduced below. The choice of the method for introduction of the nucleic acid according to the present invention and of a functionally linked nucleic acid sequence thereto encoding a gene product into plant cells is, however, not restricted to this list. Previously used methods of transformation of plants are e.g. gene transfer via *Agrobacterium tumefaciens* (e.g. dipping seeds

or leave parts into a solution of agrobacterium), with plant viruses through electroporation, microprojectile bombardment or microinjection as well as incubation of dry embryos in DNA-containing solutions and the transformation of protoplasts with the aid of polyethylenglycol. Detailed description of these methods are reviewed in e.g. Jens et al., Techniques for Genes Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, edited by Kung and Wu, Academic Press 128-143 (1993).

The nucleic acid sequence of the present invention may be used for the control of gene expression in microorganisms such as *Escherichia coli* or *Saccharomyces cerevisiae*, in monocotyledons and dicotyledons as well as in algae, animal cells and animals. Particular preferred plants thereby are crop plants such as soy, rice, cotton, sugarbeet, canola, turnip rape, sunflower, flax, cannabis, potato, tobacco, tomato, lettuce, pea, bean, carrot, onion, the different tree, nut and wine species, furthermore cereals such as barley, wheat, rye, oat, triticale, maize, edible grasses, furthermore fruit such as mango, apple, peach, gooseberry, currant, banana, melon, pumpkin and various citrus fruits such as lemon, orange, grapefruit, tangerine. The plants, transformed with the nucleic acid sequence according to the invention, may be unmodified wild-type plants or plants obtained by breeding or modified plants such as trangenic plants. The nucleic acid sequence of this invention may be used furthermore not only in plants or plant tissues, but also in single plant cells, such as in a cell culture.

The present invention furthermore refers to a transformed cell, particularly a transformed plant cell or a transformed plant tissue, in which the nucleic acid sequence according to the invention and the functionally linked nucleic acid sequence therewith encoding a gene product are stable integrated. Furthermore, the present invention relates to a plant cell transformed with the nucleic acid sequence of the invention or a transformed plant tissue, which may be regenerated to a seed producing plant. Particularly, the present invention furthermore relates to a plant which is obtainable by the method of the present invention. Furthermore, the present invention relates to seed, which is obtained from plants, which are obtained by the method according to the invention.

The present invention furthermore refers to transgenic plants with a stable in the genome integrated seed-specific regulatory nucleic acid sequence according to SEQ ID NO:1, or a fragment or derivative or homolog with the biological function of a seed-specific promoter, and a

nucleic acid sequence functionally linked to this nucleic acid sequence, encoding a gene product according to the above mentioned and described examples for such genes.

The present invention also relates to the genomic sequence or the cDNA sequence of the TT1-gene of *Arabidopsis thaliana* ecotyp columbia, which is responsible for the formation of flavonoides via intermediates. The nucleic acid sequence of the present invention is listed in SEQ ID NO:2 and 4. Furthermore, of the present invention refers to a fragment or derivative of the nucleic acid sequence according to SEQ ID NO:2 or 4 or a nucleic acid sequence, which hybridizes with the nucleic acid sequence according to SEQ ID NO:2 or 4 and is responsible for the formation of flavonoids. Preferred is a nucleic acid sequence, which hybridizes under stringent conditions with the nucleic acid sequence according to SEQ ID NO:2 or 4. The present invention relates also to the amino acid sequence of the TT1-gene of *Arabidopsis thaliana* ecotyp columbia according to SEQ ID NO:3.

The nucleic acid sequence according to the present invention may be useful for e.g. the identification and isolation of genes homologous to the TT1-gene from other organisms or of homologous genes from *Arabidopsis thaliana* using special methods of hybridization and screening, e.g. as a probe for screening DNA libraries with the help of hybridization to single stranded nucleic acids with a similar base sequence.

The present invention relates to a method of producing organisms or cells, particularly of plants, with a modified flavonoid content, comprising the stable integration of at least one nucleic acid sequences according to SEQ ID NO:2 or 4 or a homologue nucleic acid sequence, or fragment or derivative thereof in the genome of cells, particularly of plants cells or plant tissues and regeneration of the resulting plant cells or plant tissues to plants.

The nucleic acid sequence of this invention or fragment or derivative thereof may be of natural origin or artificially created. Said nucleic acid sequence or fragment or derivative thereof may be used in sense or antisense orientation. Furthermore, the nucleic acid sequence of this invention or fragment or derivative thereof may be integrated in the genomic locus of the TT1-gene or of a gene homologous to the TT1-gene from *Arabidopsis thaliana* or in a genomic locus of a gene homologous to the TT1-gene from a different plant by homologous recombination. In addition the nucleic acid sequence may be used in form of a ribonucleic acid e.g. as ribozyme. In this case

the thymine bases (T) are replaced by uracile bases (U). The formation of flavonoids may be enhanced as well as reduced by the nucleic acid sequence of the invention.

The nucleic acid sequence of the present invention may be used for example for expression in microorganisms such as *Escherichia coli* or *Saccharomyces cerevisiae*, in monocotyledons and dicotyledons as well as in algae, animal cells and animals. Particular preferred plants are crop plants such as soy, rice, cotton, sugarbeet, canola, turnip rape, sunflower, flax, cannabis, potato, tobacco, tomato, lettuce, pea, bean, carrot, onion, the different tree, nut and wine species, furthermore cereals such as barley, wheat, rye, oat, triticale, maize, edible grasses, furthermore fruit such as mango, apple, peach, gooseberry, currant, banana, melon, pumpkin and various citrus fruits such as lemon, orange, grapefruit, tangerine. The plants, transformed with the nucleic acid sequence according to the invention, may be unmodified wild-type plants or plants obtained by breeding or modified plants such as transgenic plants. The nucleic acid sequence of this invention may be used furthermore not only in plants or plant tissues, but also in single plant cells, such as in a cell culture.

Expression of the nucleic acid sequence according to the present invention may be achieved by combination of the sequence with a suited promoter. The promoter available in this combination may be the TT1 promoter according to SEQ ID NO:1 as well as a different endogenous promoter of the transformed cell or exogenous promoter from the vector. A suitable promoter is thereby in principle any regulatory sequence, which may regulate the expression of foreign genes in cells, particularly in plants, e.g. the CaMV 35S-promoter of the cauliflower-mosaic-virus (Franck et al., Cell 21, 285-294 (1980)). Expression of the nucleic acid sequence of this invention may also be achieved by a chemical inducible promoter. Examples for chemical inducible promoters are the PRPI-Promoter (Ward et al., Plant Molecular Biology 22, 361-366 (1993)), a salicylic acid inducible promoter (WO 95/19443), a benzenesulfonamide inducible promoter (EP-A 388186), a tetracyclin inducable promoter (Gatz et al., Plant Journal 2, 397-404 (1992)), a abscisic acid inducible promoter (EP-A 335528) as well as a ethanol or cyclohexanon inducible promoter (WO 93/21334). Depending on the desired location of expression also promoters may be used, which are active in certain plant tissues or plant parts. Examples for corresponding promoters are the phaseolin promoter (US 5504200), the isoflavon reductase promoter (US 5750399), a seed specific promoter from tobacco (US 5824863) or the ST-LSI promoter from potato (Stockhaus et al., EMBO Journal 8, 2445-2452 (1989)).

Furthermore, the nucleic acid sequence according to the invention may be combined with sequences, which permit a targeting in certain compartments of the plant, e.g. for transit peptides or parts of coding sequences thereof. In addition sequences coding for enzymatically active or antigenetically effective proteins such as His-tag may be combined with the above mentioned nucleic acid sequence of the invention.

Furthermore, the nucleic acid sequence of the present invention may be used in vectors, expression systems or plants, plant tissues or plant cells or animal cells or microorganisms for modifying the expression pattern.

The nucleic acid sequence according to the invention may in addition be usable in combination with various promoters for the manipulation of phenotypical and genotypical characteristics of different plants or plant tissues, such as modification of seed color, e.g. for aesthetic improvement of various plants. Ornamental plants with e.g. a knock out or mutated TT1 gene may represent optical attractive varieties.

The nucleic acid sequence of the present invention may be also used for the enhancement of the UV protection by the seed coat by a modification of the flavonoid content. Flavonoids show a absorption maxima in the ultraviolet range. The delocalized π–electrons of the phenolic groups act absorbent. An increase of the flavonoid concentration in the seed may cause a drastically reduction of UV induced tissue damage. In this way e.g. the germination rate of the seed may be improved, especially in regions with intensive sun radiation

Furthermore, the nucleic acid sequence of the present invention may be used for enhancement of the protection function of the seed coat against pathogen attack by modification of the flavonoid content. Proanthocyanidines and other phenolic components act as fungicides, among other things due to enzyme-inhibiting characteristics (Jambunathan et al., Journal of Agricultural and Food Chemistry 34, 425-429 (1986)). A concentration of this material in the seed coat may increase the pathogen resistance.

In addition, the nucleic acid sequence of the present invention may be used for the improvement of the taste quality of the seed and of other plants due to a modification of the flavonoid content,

particularly the content of condensated tannins. Condensated tannins have a big influence on the taste of many fruits and vegetables such as apple, kiwi or banana. On plant extract based drinks such as coffee, tea, wine and fruit juices are also affected in their taste quality by tannins.

Moreover, by using the nucleic acid sequence of the invention the digestibility and nutrition value as well as the quality of the protein fraction of the seed may be improved. Thereby the modified flavonoid metabolism may influence the amount and the composition of contaminating secondary ingredients. Due to the reduced portion of phenolic components and antinutritive substances purer as well as optically more attractive, lighter protein fractions are yielded of the seed and the raw fiber content may be reduced.

Furthermore, the nucleic acid sequence according to the invention may be useful as optically genetic label in plant breeding because of the ostentatious yellow seeds. In particular it is suited as a breeding accompanying marker gene for the labeling of plants and seed with further, phenotypical not visible modifications for the eased differentiation of e.g. transgenic and not-transgenic genotypes.

A further use of the nucleic acid sequence of the present invention represents the improvement of the processing ability of seed in industrial production processes. For example, condensed tannins in the testa of barley seed cause unwanted precipitates during the beer brewing process (Shirley, Seed Science Research 8, 415-422 (1998)). Due to reduced tannin content the formation of precipitates may be prevented.

The present invention relates furthermore to a transformed cell, particularly a transformed plant cell or a transformed plant tissue, in which the nucleic acid sequence according to SEQ ID NO:2 or 4 or a thereto homologous nucleic acid sequence, or fragment or derivative thereof is stable incorporated. In addition, the present invention relates to a plant cell or plant tissue transformed with the nucleic acid sequence of the invention, which is regenerable to a seed-producing plant. Particularly, the present invention relates to a plant, which is obtainable by the method of this invention. Furthermore, the present invention relates to seed, which is obtained by plants, which are produced according to the method of the invention.

The present invention relates also to transgenic plants with a stable in the genome integrated

nucleic acid sequence according to SEQ ID NO:2 or 4 or a thereto homologous nucleic acid sequence or fragment or derivative thereof with a biological activity of a polypeptid encoded by the nucleic acid sequence according to SEQ ID NO:2 or 4, and where necessary with a nucleic acid sequence functionally linked to said nucleic acid sequence according to the listed and described examples for such promoters.

EXAMPLES

The present invention is explained in more detail below by way of the examples. However, the intention is not intended to be limited thereby as the examples are merely illustrative.

Example 1: General cloning procedures

The cloning steps performed within in the scope of the present invention such as restrictions, agarose gelelectrophorese, purification of nucleic acid fragments, transfer of nucleic acids onto filters transformation and growing of bacterial cells etc. were performed as described by Sambrook et al., Molecular Cloning, Cold Spring Harbour Laboratory (1989), ISBN 0-87969-309-6.

Example 2: Production of a knockout population of *Arabidopsis thaliana*

The present invention was achieved by screening of a transposon En-1/Spm (Pereira et al, EMBO Journal 5, 835-841 (1986)) mutagenized knockout population of plants from the species *Arabidopsis thaliana* ecotyp columbia. The integration of the transposable elements in the genes of the mother plant often leads to a loss of the corresponding gene function and in many cases to a phenotype different from the wild-type of the concerned plant. For the set-up of the knockout population the autonomous En-1 element of *Zea mays* was transferred by *Agrobacterium tumefaciens* in Arabidopsis. The corresponding transposon tagging system is described in Cardon et al., Plant Molecular Biology 23, 157-178 (1993). The used Ti-Plasmid, pGV3850HPT::pkEn2, comprised the complete En-1 element as integrate. For the selection of hygromycin-resistant transformants this vector carries the HPT-gene under control of the viral CaMV 35 promoter. Seeds of a transformant with a single T-DNA insertion were sawn on a hygromycin containing medium. Seeds of plants resistant against hygromycin obtained in this way (T_2-generation) were sawn afterwards on kanamycin containing medium. In the plants selectioned like this (T_3-generation) the En-1 element was transponed out of the T-DNA. By the

means of PCR the plants of the T_4-generation were identified, which carried one or more transposed En-1 elements but no En-1 elements in the T-DNA. These plants still possessed the T-DNAs without incorporated En-1 elements. For the creation of En-1 positiv, but T-DNA negative plants, the T_4-generation was crossed with the wild-type *Arabidopsis thaliana,* ecotyp columbia and En-1 positive, T-DNA negative plants (S_0-generation) identified by PCR. Seeds of each of these plants were reproduced over 6-12 generations (until S_6- resp. S_{12}-generation), until overall 3.000 lines with totaling 15.000 independent En-1 insertions were available (Wisman et al., Plant Molecular Biology 37, 989-999 (1998), Baumann et al., Theoretical and Applied Genetics 97, 729-734 (1998)).

Example 3: Screening for TT-mutants

For the identification of phenotypical obstentatious mutants of the obtained En-1 population 2.000 families of S_6- generation (with 20 individuals) were screened for distinctive features per eye. Thereby one line (5K69) could be identified, which showed a different color of the seed coat (yellow instead of dark brown) while the production of anthocyanins in stem and leaves was obviously not affected.

The restriction of the phenotype to the seed was described by Koornneef, supra, and Shirley, supra, also for the classical tt1-mutant. To verify, if the line found in the En-population is an allele of tt1, both lines were crossed. The progeny of this cross produced again yellow seeds. This indicated that indeed both parents carry a defect allele of the TT1 gene and did entail it.

For cloning of a gene, parts of the DNA were determined, which flank a known DNA sequence, e.g a transposon. Therefore it has to been shown first, that the line 5K90 still had the En-Transposon in the TT1 gene. A population of 51 sister plants of the tt1-En line was analyzed by southern blotting for this purpose. 19 of these plants produced yellow and 33 dark brown seeds. Different bands, which hybridized with the En-probe were identified. One of this bands were found in all plants which produced yellow seeds as well as in 16 of the dark brown ones. It was not found in the remaining 17 plants. This result confirms the assumption, that all yellow seed producing plants are homozygous for an insertion in the En-Transposon at the tt1 locus while the plants with dark brown seeds are either heterozygous for this insertion or homozygous for the wild-type.

The flanking DNA of this insertion was gained by rapid amplification of genomic ends (RAGE), cp. Cormack und Somssich, Genes 194, 273-276 (1997), and cloned into the pCR-TOPO vector (Invitrogen). The resulting plasmid is pCR-RAGE. The insert of pCR-RAGE was used as probe to screen the IGF-BAC library (Mozo et al., Plant Journal 16, 377-384 (1998)). 5 positive clones were thereby identified (11O6, 3N5, 2B22, 10P4, 4M12). All of these clones are localized approximately at 55 cM on chromosom I of the *Arabidopsis thaliana* genome, which suits to the map position of the tt1-mutation.

Example 4: Sequencing of the genomic TT1-region

A 12 kb SpeI fragment of BACs 3N5, which hybridizes with said probe, was subcloned into pSK Bluescript (Stratagene). The resulting plasmid is pSK-TT1. pSK-TT1 was sequenced with the Genome Priming Systems GPS1 of New England Biolabs and a sequencer of the company ABI, model 377. The sequences were assembled and analyzed by various computer programs. Comparison of TT1 with sequences in the NCBI GenBank showed similarities with zinc finger proteins. Zinc finger proteins are capable to bind to DNA-sequences and to exert regulatory functions with regard to the expression of certain genes. The protein sequence of 303 amino acid length deduced from the TT1-cDNA shows similarities of slightly above 30 % to zinc finger proteins like StPCP1 (X82328, Kühn and Frommer 1995, MGG 247, 759-763) and ZmID1 (AF0058757, Colasanti et al, 1998, Cell 93, 593-603). A by far higher similarity of above 70% exist to database entries, which represent only hypothetical proteins until now. The comparison by computer shows beside the TT1 amino acid sequence hypothetical amino acid sequences of *Arabidopsis thaliana*, which were deduced from sequences with the acc.no AL049660, AB025629 or AC006085.9 and in case of *Hordeum vulgare* from AJ2347041. The similarities in this case extend over an explicit bigger region of the sequence, and extend over the zinc finger region.

Example 5: Determination of the TT1 cDNA

The existence of an expressed gene as well as the position of the putative introns were examined by RT-PCR. The overall length of the entire cDNA was determined by 3' and 5' race.

Example 6: Complementation of the tt1 mutation

To show that the cloned gene is in fact TT1, the mutation was complemented. Therefore the 12 kb insert from pSK-TT1 was cloned into the SpeI restriction site of the vector pGPTV-Kan-

TATA::GUS. This vector emerged out of pGPTV-Kan (Becker et al., Plant Molecular Biology 20, 1195-1197 (1992)) by exchange of the CaMV 35S promoters against a polylinker. After transformation into the agrobacterium strain CaV 3101 (with the virulence plasmid pMP90, Koncz and Schell, Molecular and General Genetics 204, 383-396 (1986)) tt1 plants were transformed by vacuum infiltration (Bechthold et al., Molecular Biology and Genetics 316, 1194-1199 (1993)). Transformants were selected on kanamycin containing MS medium and analyzed due to their seed color. Seeds of complemented plants correspond to the wild-type regarding the coloration.

Example 7: Determination of the cellular localization of the TT1 protein

To determine the cellular localization of the TT1 protein, it was fused to the „green fluorescent protein" (GFP) with the c-terminal end. Using pAVA393 (by Arnim et al., Genes 221, 35-43 (1998)) and the complete cDNA pTT1-GFP was developed. The plasmid was transfected in Arabidopsis protoplasts (Hartmann et al., Plant Molecular Biology 36, 741-54 (1998) and after an incubation period of 20 hours the GFP-fluorescence was determined. The localization of the TT1 protein in the nucleus could be demonstrated by this examination.

Example 8: Expression analyses

For the examination of the TT1 promoter a 3 kb fragment was first incorporated into the vector pBT10 (Feldbrügge et al., Plant Journal 11, 1079-1093 (1997)) in front of GUS and then the whole promoter-GUS cassette was transferred in the binary vector pGPTV. After transformation into Agrobacterium, wild-type plants of *Arabidopsis thaliana* Columbia were infiltrated. Transformants were selected on kanamycin containing agar plates. After approximately ten days the germ buds were transferred to soil and cultivated in the greenhouse by long day conditions until seed maturation. Activity of the reporter enzyme β-glucuronidase was shown by a change from colourless x-gluc to a blue marker. Therefore the substrate was infiltrated into the germ buds, leaves and inflorescences with siliques of various developmental stages and afterwards the chlorophyll was removed out of the tissue by ethanolic extraction (figure 8).

After an incubation period of two days in the substrate solution the reporter gene activity in the transgene *TT1*-GUS-plants was shown. While under this conditions no blue staining could be seen in the wild-type, GUS-activity was noticed in the opened flowers and the developing siliques in the transgene plants. In opened flowers the gynoeceum tops the anthers (figure 8A

and B) and pollination was already done. The examination of the plant material by light microscopy showed that the GUS activity was demonstrated in the funiculi and integuments of the ovules as well as in other maternal tissues (septum, basis of silique) (figure 8 C and D). It was above all demonstrated in the topmost and lowest ovules of young siliques (figure 8 B and E). GUS-activity could be demonstrated in siliques until stage 17, but not in older ones.

SEQUENZPROTOKOLL

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<120> Plants with modified gene expression

<130> GI-001

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CLAIMS

1. A method for producing a plant with modified gene expression, comprising stable integration of a seed specific regulatory sequence or a fragment or derivative thereof, provided said fragment or derivative controls specifically the expression of genes in the seed, and a nucleic acid sequence encoding a gene product wherein the nucleic acid sequence is functionally linked to said seed specific regulatory sequence or the fragment or derivative thereof, into the genome of plant cells or plant tissues and regeneration of the obtained plant cells or plant tissues to plants.

2. Method according to claim 1, wherein said gene expression is enhanced or reduced.

3. Method according to claim 1 or 2, wherein for said nucleic acid sequence encoding a gene product an endogeneous or exogeneous nucleic acid sequence is used.

4. Method according to anyone claims 1-3, wherein for said nucleic acid sequence encoding a gene product a nucleic acid sequence selected from the group of genes of the phenyl propanoid metabolism, seed specific genes, seed coat-specific genes or genes of the general metabolism is used.

5. Method according to claim 4, wherein for said genes of the phenylpropanoid metabolism a nucleic acid sequence selected from the group of genes for phenylalanine ammonia-lyase, cinnamate 4-hydroxylase, 4-coumarate-coA ligase, chalcone synthase, chalcone isomerase, chalcone reductase, flavanone 3-hydroxylase, flavonoid-3'-hydroxylase, flavonoid-3'5'-hydroxylase, dihydroflavono-4-reductase, leucoanthocyanidin reductase, leucoanthocyanidin dioxygenase, 3'-glucosyltransferase, 5'-glucosyltransferase and O-methyl transferase.

6. Method according to claim 4, wherein for said seed-specific genes a nucleic acid sequence is used selected from the group of genes influencing germ tendency or dormancy, or pathogen resistance, or the TT1 gene according to SEQ ID NO:2 and SEQ ID NO:4.

7. Method according to claim 4, wherein for said genes of the general metabolism a nucleic acid sequence is used selected from the group of genes for ADP glucose synthethase, starch synthase, ADP glucose pyrophosphorylase and yeast invertase.

8. Method according to anyone of claims 1 to 7, wherein for said seed-specific regulatory sequence the nucleic acid sequence according to SEQ ID NO:1 or a fragment or derivative thereof is used.

9. Transformed plant cell or transformed plant tissue, characterised in that a seed specific regulatory sequence or a fragment or derivative thereof and a nucleic acid sequence encoding a gene product wherein the nucleic acid sequence is functionally linked to said seed specific regulatory sequence or a fragment or derivative thereof is stable incorporated into the genome of the plant cell or the plant tissue.

10. Nucleic acid sequence according to SEQ ID NO:1.

11. Fragment or derivative of the nucleic acid sequence according to claim 10 or a nucleic acid sequence which hybridizes with the nucleic acid sequence according SEQ ID NO:1 and is responsible for the seed specific expression.

12. Nucleic acid sequence according to claim 11, wherein the hybridizing nucleic acid sequence hybridizes with the nucleic acid sequence according SEQ ID NO:1 under stringent conditions.

13. Method for producing plants with modified flavonoid content, comprising stable integration of at least of the nucleic acid sequence according to SEQ ID NO:2 or 4 or a homologous nucleic acid sequence thereto, or of a fragment or derivative thereof with the biological activity of a polypeptide encoded by the nucleic acid sequence according to SEQ ID NO:2 or 4, into the genome of plant cells or plant tissues and regeneration of the obtained plant cells or plant tissues to produce plants.

14. Method according to claim 13, whereby the integrated nucleic acid sequence or a

fragment or derivate thereof is expressed in sense or antisense orientation compared to the endogenous nucleic acid sequence.

15. A method according to claim 13 or 14, wherein the formation of flavonoids is inhibited by a ribozyme, comprising the integrated nucleic acid sequence or a fragment or derivative thereof.

16. Method according to claim 13 or 14, wherein the nucleic acid sequence or a fragment of derivative thereof is integrated into the genomic region of the homologous endogenous gene by homologous recombination.

17. Method according to anyone of claims 13 to 16, wherein the nucleic acid sequence or a fragment or derivative thereof is functionally linked to a regulatory DNA sequence, which controls the expression of the integrated nucleic acid sequence or a fragment or derivative thereof.

18. Method according to claim 17, wherein the regulatory DNA sequence is selected from the group of promoters CaMV 35S Promoter, PRPI promoter, phaseolin promoter, isoflavon reduktase promoter, ST-LSI promoter, salicylic acid-inducible promoter, benzenesulfonamide-inducible promoter, tetracycline-inducible promoter, abscisic acid-inducible promoter, ethanol- or cyclohexanon-inducible promoter, promoter according to SEQ ID NO:1 or a seed specific promoter from tobacco.

19. Nucleic acid sequence according to SEQ ID NO:2 or 4.

20. Fragment or derivative of the nucleic acid sequence according to SEQ ID NO:2 or 4, or a nucleic acid sequence which hybridizes to the nucleic acid sequence according to SEQ ID NO:2 or 4 and is responsible for the formation of flavonoids.

21. Nucleic acid sequence according to claim 20, wherein the hybridizing nucleic acid sequence hybridizes under stringent conditions with the nucleic acid sequence according to SEQ ID NO:2 or 4.

22. Transformed plant cell or transformed plant tissue, characterised in that the nucleic acid sequence according to anyone of claims 19 to 21 is stable integrated into the genome of the plant cell or plant tissue.

23. Amino acid sequence as listed in SEQ ID NO:3.

24. Plant cell or plant tissue according to claim 9 or 22, regenerable to a seed producing plant.

25. Plant obtainable according to anyone of claim 1 to 8 or 13 to 18.

26. Seeds obtained by plants according to claim 25.

27. Vector, comprising a nucleic acid sequence according to anyone of claims 10 to12 or 19 to 21.

28. Transgenic plant with a stable into the genome integrated seed specific regulatory nucleic acid sequence according to SEQ ID NO:1, or a fragment or derivative or homolog thereof with the biological function of a seed specific promoter, and a nucleic acid sequence encoding a gene product functionally linked to said seed specific regulatory nucleic acid sequence.

29. Transgenic plant with a stable into the genome integrated nucleic acid sequence according to SEQ ID NO:2 or 4 or a homologous nucleic acid sequence thereto, or a fragment or derivative thereof with the biological activity of a polypeptide encoded by the nucleic acid sequence according to SEQ ID NO:2 or 4.

30. Transgenic plant according to claim 29, wherein the nucleic acid sequence or a fragment or derivative thereof is functionally linked to a regulatory DNA sequence, which controls the expression of the integrated nucleic acid sequence or a fragment or derivative thereof.

Abstract

The present invention relates to a method for producing a plant with modified gene expression, comprising the stable integration of a seed-specific regulatory sequence or fragment or derivative thereof and a nucleic acid sequence that is functionally linked to said seed-specific regulatory sequence or fragment or derivative thereof, encoding a gene product, in the genome of plant cells or plant tissues, and the regeneration of the resulting plant cells or plant tissues to plants. The present invention also relates to a method for producing plants with a modified flavonoid content, comprising the stable integration of at least the nucleic acid sequence according to SEQ ID NO:2 or 4 or a nucleic acid sequence that is homologous with this, or fragment or derivative thereof in the genome of plant cells or plant tissues, and the regeneration of the resulting plant cells or plant tissues to plants.

FIG. 1 A

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FIG. 1 B

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3001 ccccaagattcccatttactcacttctctatttggtacgtaagtcacaca
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3101 tcgactttcgtaaacctatatagttcatccaatattattcggtaaattcg
3151 atatttatcaatctttattctcgtaggttaaaggagacgattgatacgtg
3201 ggatctacttacgtatctgcatgattattagttataaaagttattgcaaa
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3351 atttcatctctctccaacaactattcaccacattcaATG
```

FIG. 2

```
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3651 ATCGGCCTTCCTGGTTTTGGTAAACCAAGCAATGATGCTAAACAGCTGAA
3701 GAAGAGAAATGGGAAGGAGATCGCCACATATGACGCCGGAAAAGGCATCG
3751 AGAATGAACTTTCCGGAAAGGCATACTGGATCCCGGCGCCGGAGCAAATT
3801 CTCATAGGGTTCACTCATTTTTCTTGCCATGTATGCTTCAAGACATTCAA
3851 TCGCTACAACAATCTTCAGgtacgagtcaatatatctcatgcgcattgct
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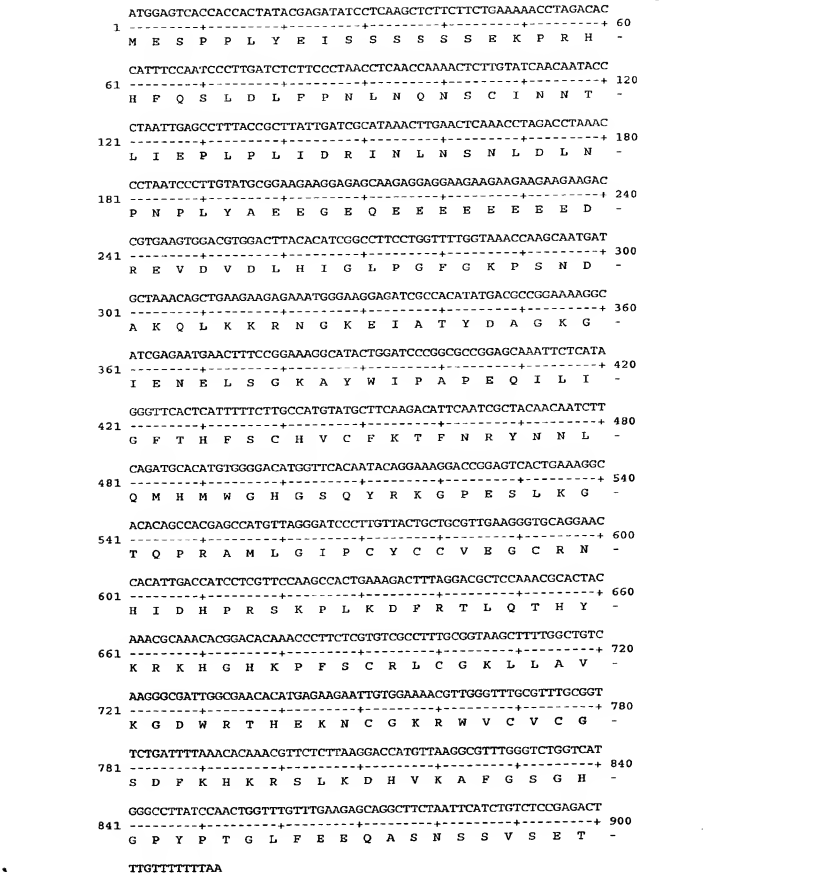
FIG: 3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H F Q S L D L F P N L N Q N S C I N N T -
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L I E P L P L I D R I N L N S N L D L N -
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P N P L Y A E E G E Q E E E E E E E E D -
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R E V D V D L H I G L P G F G K P S N D -
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I E N E L S G K A Y W I P A P E Q I L I -
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G F T H F S C H V C F K T F N R Y N N L -
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S D F K H K R S L K D H V K A F G S G H -
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G P Y P T G L F E E Q A S N S S V S E T -
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L F F * -

FIG. 4

```
               1                                                    50
AtTT1          .......... .......... .......... .......... ..........
AtAL049660     .......... .......... .......... .......... ..........
AtAB025629     MLFSTVLSHR TLYILTCPNT LIHSYTHPHI HAYLAFTGFL TQLHHLEISC
AtAC006085.9   .......... .......... .......... .......... ..........
Hv234704       .......... .......... .......... .......... ..........
Consensus      .......... .......... .......... .......... ..........

               51                                                  100
AtTT1          .......... .......... .......... .....MESPP LYEISSSSSS
AtAL049660     ......MTDP YSNFFTDWFK SNPFHH..YP NSSTNPSPHP LPPVTPPSSF
AtAB025629     LLLLFFSLSS LLKLMADPDC IFRNGYVDYY NYSFNYATSL SRIYNSHDSF
AtAC006085.9   .......... ....MSNPAC SNLFNNGCDH N.SFNYSTSL SYIYNSHGSY
Hv234704       .......... .......... .......... .......... ..........
Consensus      .......... .......... .......... .......... ........s.

               101                                                 150
AtTT1          EKPRHHFQSL DLFPNLNQNS CINNTLIEPL PLIDRINLNS NLDLNPNP..
AtAL049660     FFFPQSGD.. LRRPPPPPTP PPSPPLREAL PLLSLSPANK QQDHHHNH.D
AtAB025629     YYPHQTTNPN INE.NPNLTS PDSPPLREAL PLLSLSPIHK HQEPTANHHE
AtAC006085.9   YYSNTTNPNY INHTHTTSTS PNSPPLREAL PLLSLSPI.R HQEQQDQH..
Hv234704       .......... .......... .......... .......... ..........
Consensus      .......... .......... .....l.e.l pl........ ..........

               151                                                 200
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AtAL049660     HLIQEPPSTS MDVDYDHHHQ DDHHNLDDDD HDVTVALHIG LPSPSAQEMA
AtAB025629     YYFMETTETS SNSNFLDQCQ DSYR...... HDVTVDLHLG LPNLGDGG..
AtAC006085.9   .YFMDTHQIS S.SNFLDDPL .......... ..VTVDLHLG LPNYGVGE..
Hv234704       .......... .......... .......... .......... ..........
Consensus      .......... .......... .......... ..v.v.lh.g lp........

               201                                                 250
AtTT1          .....KPSND AKQLKKRNGK EIATYDAGKG IENELSGKA. ..........
AtAL049660     SLLMMSSSSS SSRTTHHHED MNHKKDLDHE YSHGAVGGGE DDDEDSVGGD
AtAB025629     .....SSSSD VVLDSTDHQE GHHDHHQDQG LEVTMAS... DHDDEHGGLQ
AtAC006085.9   .....SIRSN IAPDATTDEQ ...DQDHDRG VEVTVESHLD DDDDHHGDLH
Hv234704       .......... .......... .......... .......... ..........
Consensus      .......... .......... .......... .......... ..........

               251                                                 300
AtTT1          .......... ..YWIPAPEQ ILIGFTHFSC HVCFKTFNRY NNLQMHMWGH
AtAL049660     GGCRISRLNK GQYWIPTPSQ ILIGPTQFSC PVCFKTFNRY NNMQMHMWGH
AtAB025629     RGNHLHH... ..FWIPTPSQ ILMGPTQFSC PLCFKTFNRY NNMQMHMWGH
AtAC006085.9   RG...HH... ..YWIPTPSQ ILIGPTQFTC PLCFKTFNRY NNMQMHMWGH
Hv234704       .......... .......... .......... .......... ...QMHMWGH
Consensus      .......... ...wip.p.q il.g.t.f.c ..cfktfnry nn.QMHMWGH

               301                                                 350
AtTT1          GSQYRKGPES LKGTQPRAML GIPCYCCVEG CRNHIDHPRS KPLKDFRTLQ
AtAL049660     GSQYRKGPES LRGTQPTGML RLPCYCCAPG CRNNIDHPRA KPLKDFRTLQ
AtAB025629     GSQYRKGPES LRGTQPTAML KLPCYCCAPG CKNNIDHPRA RPLKDFRTLQ
AtAC006085.9   GSQYRKGPES LRGTQPTGML RLPCFCCAPG CKNNIDHPRA KPLKDFRTLQ
Hv234704       GREYRKGPES LKGTQTVALL KVPCYCAA.G CRNSVSHPRA RPLKDFRT..
Consensus      Gs#YRKGPES LkGTQp.a$L k.PCYCca.G CrN.!dHPRa rPLKDFRTlq

               351                                                 400
AtTT1          THYKRKHGHK PFSCRLCGKL LAVKGDWRTH EKNCGKRWVC VCGSDFKHKR
AtAL049660     THYKRKHGIK PFMCRKCGKA FAVRGDWRTH EKNCGKLWYC ICGSDFKHKR
AtAB025629     THYKRKHGVR PFACRRCGKA FAVKGDWRTH EKNCGKLWYC SCGSDFKHKR
AtAC006085.9   THYKRKHGSK PFACRMCGKA FAVKGDWRTH EKNCGKLWYC SCGSDFKHKR
Hv234704       .......... .......... .......... .......... ..........
Consensus      thykrkhg.. pf.cr.cgk. .av.gdwrth ekncgk.w.c .cgsdfkhkr

               401                                             448
AtTT1          SLKDHVKAFG SGHGPYPTG. .LFEEQASNS SVSETLFF.. ........
AtAL049660     SLKDHIKAFG NGHGAYGID. .GFDEED..E PASEVEQLDN DHESMQSK
AtAB025629     SLKDHVKAFG NGHVPC.... CGIDHEEE.E AASDVEQQE. ........
AtAC006085.9   SLKDHVKAFG NGHVPCGIDS FGGDHEDYYD AASDIEQ... ........
Hv234704       .......... .......... .......... .......... ........
Consensus      slkdh.kafg .gh....... .......... ..s....... ........
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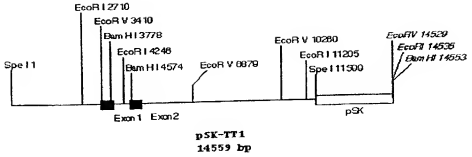
FIG. 5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pSK-TT1
14559 bp

FIG. 6

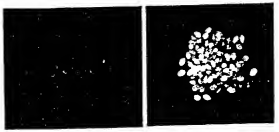
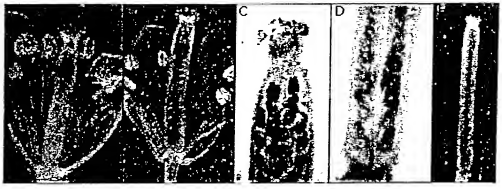


FIG. 7

Kontrolle TT1-GFP

FIG. 8



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Martin Sagasser, et al.

Serial No.: 10/030,386

Filed: January 2, 2002

For: PLANTS WITH MODIFIED GENE EXPRESSION

Group Art Unit: Unknown

Examiner: Unknown

Atty. Dkt. No.: DEBE:005US/SLH

ELECTION UNDER 37 C.F.R. §§ 3.71 AND 3.73
AND POWER OF ATTORNEY

Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned, being Assignee of record of the entire interest in the above-identified application by virtue of an assignment recorded in the United States Patent and Trademark Office as set forth below, hereby elects, under 37 C.F.R. § 3.71, to prosecute the application to the exclusion of the inventors.

The Assignee hereby revokes any previous Powers of Attorney and appoints:

Louis T. Pirkey, Reg. No. 22,393; David D. Bahler, Reg. No. 30,932; Christopher R. Benson, Reg. No. 31,941; David L. Parker, Reg. No. 32,165; Richard J. Groos, Reg. No. 32,231; Stephen D. Dellett, Reg. No. 32,564; William G. Barber, Reg. No. 33,154; Michael S. Metteauer, Reg. No. 34,875; Mark B. Wilson, Reg. No. 37,259; Daniel J. Brennan, Reg. No. 37,287; Steven L. Highlander, Reg. No. 37,642; Erik R. Nordstrom, Reg. No. 39,792; Stephen P. Meleen, Reg. No. 40,724; Robert E. Hanson, Reg. No. 42,628; Thomas M. Boyce, Reg No. 43,508; Michael C. Barrett, Reg. No. 44,523; Mark T. Garrett, Reg. No. 44,699; Gina N. Shishima, Reg. No. 45,104; Charles P. Landrum, Reg. No. 46,855; and Priya D. Subramony, Reg. No. 50,939;

each an attorney or agent of the firm of FULBRIGHT & JAWORSKI L.L.P., as its attorney or agent for so long as they remain with such firm, with full power of substitution and revocation, to prosecute the application, to make alterations and amendments therein, to transact all business in the Patent and Trademark Office in connection therewith, and to receive any Letters Patent, and for one year after issuance of such Letters Patent to file any request for a certificate of correction that may be deemed appropriate.

Pursuant to 37 C.F.R. § 3.73, the undersigned has reviewed the evidentiary documents, specifically the Assignment to MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V., referenced below, and certifies that to the best of my knowledge and belief, title remains in the name of the Assignee.

Please direct all communications as follows:

Steven L. Highlander
FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 474-5201

ASSIGNEE:
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.

By: Christa Herzog
Name: ~~Christa Herzog~~
Title: ~~Head of Patent Department~~

Date: April 19, 2002

ASSIGNMENT:
- ☒ Concurrently filed
- ☐ Previously recorded
  - Date: _____
  - Reel: _____
  - Frames: _____

3-UU

Inventor's Full Name:	Koen		Dekker
Inventor's Signature:			
Country of Citizenship:	The Netherlands	Date:	19/4/2002
Residence Address: (street, number, city, state, and/or country)	Goldammerweg 9 D-50829 Köln Germany DEX		
Post Office Address: (if different from above)			

PCT/DE00/02233	July 3, 2000	Pending
(Application Serial No.)	(Filing Date)	(Status)
(Application Serial No.)	(Filing Date)	(Status)

I hereby direct that all correspondence and telephone calls be addressed to Steven L. Highlander, Fulbright & Jaworski L.L.P., 600 Congress Avenue, Suite 2400, Austin, Texas 78701, (512) 474-5201.

I HEREBY DECLARE THAT ALL STATEMENTS MADE OF MY OWN KNOWLEDGE ARE TRUE AND THAT ALL STATEMENTS MADE ON INFORMATION AND BELIEF ARE BELIEVED TO BE TRUE; AND FURTHER THAT THESE STATEMENTS WERE MADE WITH THE KNOWLEDGE THAT WILLFUL FALSE STATEMENTS AND THE LIKE SO MADE ARE PUNISHABLE BY FINE OR IMPRISONMENT, OR BOTH, UNDER SECTION 1001 OF TITLE 18 OF THE UNITED STATES CODE AND THAT SUCH WILLFUL FALSE STATEMENTS MAY JEOPARDIZE THE VALIDITY OF THE APPLICATION OR ANY PATENT ISSUED THEREON.

1-00

Inventor's Full Name:	Martin		Sagasser
Inventor's Signature:	M. Sagasser		
Country of Citizenship:	Germany	Date:	19.04.2002
Residence Address: (street, number, city, state, and/or country)	~~Lichtstrasse 23~~ ~~D-50825 Köln~~ Germany	SCHWERINSTRASSE 8 D-50733 Köln DEX	
Post Office Address: (if different from above)			

2-00

Inventor's Full Name:	Bernd		Weisshaar
Inventor's Signature:			
Country of Citizenship:	Germany	Date:	19.04.2002
Residence Address: (street, number, city, state, and/or country)	Fingerhutweg 13 D-50226 Frechen Germany DEX		
Post Office Address: (if different from above)			

PATENT
DEBE:005US

DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or the below named inventors are the original, first and joint inventors (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **PLANTS WITH MODIFIED GENE EXPRESSION**, the Specification of which:

☐ is attached hereto.
☒ was filed on **January 2, 2002** as Application Serial No. **10/030,386**.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent, United States provisional application(s), or inventor's certificate listed below and have also identified below any foreign application for patent, United States provisional application, or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIORITY APPLICATION(S)			**Priority Claimed**
DE 199 30 570.6	Germany	July 2, 1999	YES
(Number)	(Country)	(Date Filed)	Yes/No
(Number)	(Country)	(Date Filed)	Yes/No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below or any PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to me to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56, which become available between the filing date of the prior application and the national or PCT international filing date of this application:

SEQUENCE LISTING

<110> Max-Planck-Gesellschaft z.
Ford. d. Wissenschaften

<120> Plants With Modified Gene Expression

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Asp Arg Ile Asn Leu Asn Ser Asn Leu Asp Leu Asn Pro Asn Pro Leu
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Tyr Ala Glu Glu Gly Glu Gln Glu Glu Glu Glu Glu Glu Glu Asp
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Arg Glu Val Asp Val Asp Leu His Ile Gly Leu Pro Gly Phe Gly Lys
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Pro Ser Asn Asp Ala Lys Gln Leu Lys Lys Arg Asn Gly Lys Glu Ile
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Ala Thr Tyr Asp Ala Gly Lys Gly Ile Glu Asn Glu Leu Ser Gly Lys
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Ala Tyr Trp Ile Pro Ala Pro Glu Gln Ile Leu Ile Gly Phe Thr His
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Phe Ser Cys His Val Cys Phe Lys Thr Phe Asn Arg Tyr Asn Asn Leu
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Cys Cys Val Glu Gly Cys Arg Asn His Ile Asp His Pro Arg Ser Lys
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Pro Leu Lys Asp Phe Arg Thr Leu Gln Thr His Tyr Lys Arg Lys His
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Gly His Lys Pro Phe Ser Cys Arg Leu Cys Gly Lys Leu Leu Ala Val
225                 230                 235                 240

Lys Gly Asp Trp Arg Thr His Glu Lys Asn Cys Gly Lys Arg Trp Val
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      50                  55                  60

Tyr Ala Glu Glu Gly Glu Gln Glu Glu Glu Glu Glu Glu Glu Asp
 65                  70                  75                  80

Arg Glu Val Asp Val Asp Leu His Ile Gly Leu Pro Gly Phe Gly Lys
                 85                  90                  95

Pro Ser Asn Asp Ala Lys Gln Leu Lys Lys Arg Asn Gly Lys Glu Ile
            100                 105                 110

Ala Thr Tyr Asp Ala Gly Lys Gly Ile Glu Asn Glu Leu Ser Gly Lys
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Ala Tyr Trp Ile Pro Ala Pro Glu Gln Ile Leu Ile Gly Phe Thr His
    130                 135                 140

Phe Ser Cys His Val Cys Phe Lys Thr Phe Asn Arg Tyr Asn Asn Leu
145                 150                 155                 160

Gln Met His Met Trp Gly His Gly Ser Gln Tyr Arg Lys Gly Pro Glu
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Ser Leu Lys Gly Thr Gln Pro Arg Ala Met Leu Gly Ile Pro Cys Tyr
            180                 185                 190

Cys Cys Val Glu Gly Cys Arg Asn His Ile Asp His Pro Arg Ser Lys
        195                 200                 205

Pro Leu Lys Asp Phe Arg Thr Leu Gln Thr His Tyr Lys Arg Lys His
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Gly His Lys Pro Phe Ser Cys Arg Leu Cys Gly Lys Leu Leu Ala Val
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Lys Gly Asp Trp Arg Thr His Glu Lys Asn Cys Gly Lys Arg Trp Val
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Cys Val Cys Gly Ser Asp Phe Lys His Lys Arg Ser Leu Lys Asp His
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